FINAL REPORT

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MOLECULAR GENETIC MODIFICATION OF FLAX SEED OIL

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A. SUMMARY

The high linolenic acid content in linseed oil is what makes it valuable as an industrial oil. Our goal was to develop modified flaxseed oil composition and content by molecular genetic means to enhance its economic value. Flax was transformed with two oil genes (FAD3, and SLC1) with the goal of altering linolenic acid content and/or increasing the total oil content. Transgenic flax with elevated linolenic acid; oleic acid and oil content were produced. The possible benefit of combining the two transgenes through cross-pollination was explored and lines with promising profiles were obtained.

B. TECHNICAL REPORT

BACKGROUND AND OBJECTIVES

In Western Canada, flax has traditionally been grown as an industrial oilseed crop. The high linolenic acid content in flaxseed oil is the reason for its value as an industrial drying oil. Our goal was to modify flaxseed oil composition and content by molecular genetic means to enhance its economic value.

Linolenic acid is the end product of the consecutive desaturation of the saturated fatty acid stearic acid (18:0), to oleic acid (18:1) to linoleic acid (18:2) and finally to linolenic acid (18:3). A specific desaturase gene regulates each stage in the production of linolenic acid from stearic acid. Cahoon et al. (1999 PNAS 96: 12935-12940) showed that the insertion of an extra copy of a desaturase gene in a plant can cause an over-expression of the desaturase and alter the balance of unsaturated fatty acids. Therefore, we introduced an additional copy of the Delta-15 (FAD3) desaturase gene isolated from canola and driven by a flax SAD2 promoter into an elite flax line. FAD3 desaturase controls the conversion of linoleic acid (18:2) to linolenic acid (18:3). However, introducing an extra copy of a gene is also known, in some cases, to cause overall suppression of gene expression. Therefore, all progeny were screened for low linolenic acid content as well.

An increase in seed oil content in flax would also increase its value. The native acyl transferase enzyme in flax transfers linolenic acid (an unsaturated fatty acid) to the sn-1 and sn-3 positions but not the sn-2 position on triglyceride oil molecules. The sn-2 acyl transferase gene, SLC1, isolated from yeast has the ability to put both unsaturated and saturated fatty acids at all three positions. Zou et al. (1997, Plant Cell 9: 909-923) found that Brassicaceae expressing this transgene had increased seed oil content by 10 – 25% and that the SLC1 over-expression resulted in an increase in the most abundantly available fatty acid in transgenic canola seed oil. Therefore, transgenic seed over-expressing the SLC1 gene might produce an elevated linolenic acid content as well as higher oil content. To explore this possibility SLC1 was transferred in to flax.

Our goal of an increased linolenic acid content is possible from transgenic flax with either transgene. It is possible that the effect of combining the FAD3 and SLC1 transgenes in one line could be additive and lead to the development of a more desirable phenotype. With this in mind a crossing program utilizing high linolenic acid T_1 lines from both transgenes was designed.

RESULTS AND CONCLUSIONS

RESULTS

1. Construction of Transformation Vectors with Selected Genes and Promoters

a) FAD3 (Fatty Acid Desaturase 3)

The FAD3 cassette used for this experiment was constructed from the following the components. The *Brassica napus* FAD3 gene (Δ15 desaturase gene) excised from the plasmid pBNDES3 which was obtained from the Arabidopsis Biological Resource Center (ABRC) Columbus, Ohio (Genebank accession #L01418, NID #G167147). This gene was driven by the flax stearoyl-acyl carrier protein desaturase promoter (SAD2P) previously isolated by our lab (NCBI accession # AJ006956 bases 1-1182). The terminator used was the Nopaline synthase (NosT) (Appendix 1) from *Agrobacterium tumefaciens*, which was excised from clone pCDC 201 (Figure 1, originally derived from pCaMVCN, Pharmacia, Peapack, NJ). All DNA fragments were purified by agarose gel electrophoresis separation.

To isolate the NosT from pCDC 201 it was cut using BamH I and Xba I. The products were run on a 2% TAE (Tris-Acetate-EDTA) agarose gel to isolate the 280-bp (NosT) fragment. The isolated fragment was extracted using the Boerhringer Mannheim's agarose gel DNA extraction kit (Indianapolis, IN).

The plasmid pCDC 105 (Appendix 2, Figure 2) containing the SAD2P was linearized using BamH I and Xba I, and a ligation with the isolated NosT was conducted to produce pCDC 1051 (Figure 3). The pCDC 1051 clones with the tandem integration of the SAD2P and NosT fragments were confirmed by gel electrophoresis and restriction digests.

The map supplied by the ABRC for the multiple cloning sites (MCS) of pBNDES3 was discovered to be erroneous. A modified and updated map of the MCS was produced through restriction analysis and partial sequencing of the 5' and 3' flanks of the FAD3 gene fragment (Appendices 3, 4) and the correct restriction map was constructed (Figure 4).

The *Brassica napus* FAD3 gene is 1.35 Kb in size (Arondel et al. 1992, Science 258: 1353-1355). To isolate the gene the pBNDES3 was digested with EcoR I and the FAD3 gene fragment was isolated by gel electrophoresis. It was then gel purified using the same procedures used for the promoter. The FAD3 gene was ligated downstream of the SAD2P at the EcoR I site (Figure 5) of the pCDC 1051 plasmid to produce pCDC 1052 (Figure 6). The correct orientation and order of the cassette was confirmed by restriction analysis.

The FAD3 cassette was excised from pCDC 1052 using Sal I and Not I restriction enzymes and then gel purified. The excised FAD3 gene cassette (Appendix 5) was directionally cloned into the pBIN19 vector (Frisch et al. 1995, Plant Mol. Biol. 27: 405-409, Figure 7) which was linearized using Sal I and Hind III. A synthetic oligonucleotide adapter containing Not I, Cla I and Hind III restriction sites was used to bridge the Not I restriction terminal of the FAD3 cassette to the Hind III restriction terminal of the pBIN19 vector (Figure 8). The adapter was synthesized from the following

oligonucleotides (5' GGC CGC ATC GAT A 3') and (5' AGC TTA TCG ATG C 3'). The resulting plasmid was labeled pVIJ1 (Figure 9, Appendix 6).

The FAD3 cassette was also mobilized into the pCAMBIA 1200 vector (Hajdukiewicz et al. 1994, Plant Mol. Biol. 25: 989-994) using the same strategy (Figure 10) and the resulting plasmid was labeled pVIJ4 (Figure 11).

b) SLC1 (Sphingolipid Compensation 1)

A *sn*-2 acyl transferase gene, *SLC1* (Sphingolipid Compensation 1), isolated from *Saccharomyces cerevisiae* by Lester et al. (1993, J. Biol. Chem. 268: 845-856, NCBI accession #L13282) was obtained from the National Research Council Plant Biotechnology Institute in Saskatoon in the vector pSLC1 (Figure 12). The *SLC1* gene cassette (Figure 13) consisting of the CaMVCN 35S promoter (429 bp), *SLC1* gene (950 bp) and Nos terminator (250 bp) was released from the vector pRD400 using the restriction enzymes EcoR I and HinD III. The cassette was then cloned into the multi-cloning sites of pBIN19 and pCAMBIA1200 using the same restriction sites. The T-DNA vectors thus obtained were named pVIJ2 and pVIJ3 respectively (Figures 14, 15).

All plasmid vectors produced for this project were subsequently mobilized into the *Agrobacterium tumefaciens* strain GV3101 bearing helper plasmid pMP90 (Koncz and Schell 1986, Mol Gen Genet 204: 383-396).

Discussion

Studies with constitutive (universal) promoters have indicated that plant wide expression of some genes (ie. herbicides, insecticides) is desirable. However, fatty acids are important membrane lipids as well as seed storage lipids. Therefore, in addition to impacting the seed oil fatty acid profile a universally expressed FAD3 desaturase transgene could have an impact on cell membranes and result in unsuitable or non-viable phenotypes. To reduce this possibility a time and tissue specific promoter, SAD2, was selected to produce the FAD3 transgenic lines.

For our experiments, the constitutive 35S CaMVCN promoter drove the SLC1 transgene, even though it was possible to obtain undesirable phenotypes. Previous experiments with Brassicacea (Zou et al.) have shown that this gene cassette did produce desirable phenotypes. No direct comparisons between the SLC1 transgene with the 35S and SAD2 promoters were made due to lack of resources.